REMARKS

Claims 1, 2, 4, 6-11, 15 and 16 are all the claims pending in the application.

Claim 1 has been amended to correct a spell error and incorporate the features of Claims 3 and 5. Accordingly, Claims 3 and 5 have been canceled. Claim 6 has been amended to change dependency according to the cancellation of Claim 5. Claims 7 and 9 have been amended to change "foreign protein" to "serine-rich protein." Claim 2 has been rewritten into an independent claim format by incorporating all limitations of Claim 1. The features of Claim 13 has been incorporated into Claim 2 and Claim 13 has been canceled accordingly.

Claims 15 and 16 are newly added and support can be found from original Claims 6 and 10, respectively.

The specification has been changed to correct grammatical errors, to insert sources of materials used in working examples, and/or to more clearly describe the invention. No new matter has been introduced. For example, support for changing the term "G-CSF" to "hG-CSF" may be found at, for example, the description of page 12 of the specification, lines 4-10.

Entry of the amendments and reconsideration are respectfully requested.

A. Objections to the Claims

Claims 1 and 10 stand objected to because the word "cysteine" of Claim 1 was misspelled and a space was missing following the word plasmid on line 2 of Claim 10.

Claims 1 and 10 have been amended to cure these clerical errors. Withdrawal of the objections is respectfully requested in view of the amendments.

B. Rejection of Claims 1-14 under 35 U.S.C. 112, second paragraph

Claims 1-14 stand rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the word "foreign protein" was pointed out by the Office Action. The Office Action also stated Claim 10 was confusing because Claim 10 referred to "vector according to claim 7" which was "selected from plasmid pAC104CysK or plasmid pEDIL-12p40," but none of the plasmid pAC104CysK or plasmid pEDIL-12p40 had the limitations recited in the vector of Claim 7.

Claims 1, 2, 7 and 9 have been amended to change the word "foreign protein" to "serine-rich protein" and to clarify that the serine-rich protein is introduced to a host cell using a vector containing a gene encoding the serine-rich protein. Claim 10 has been amended to recite that the vector of Claim 7 comprises both plasmid pAC104CysK and plasmid pEDIL-12p40.

Therefore, the rejections under 35 U.S.C. § 112, second paragraph becomes moot and it is respectfully requested that the rejection be withdrawn.

C. Rejection of Claim 10 under 35 U.S.C. § 112, first paragraph

Claim 10 stands rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Office Action's position is that the vectors used in the present application, which are essential to the claimed invention and appear to be novel, are not described in the specification in a way that they are repeatable.

Plasmid pAC104CysK as shown in Fig. 2 and plasmid pEDIL-12p40 as shown in Fig. 3 are constructed from known vectors and sequences. *E. coli* BL21(DE3) (Example 2) is

commercially available from Novagen, Inc. (Madison, Wis); plasmid pACYC184 (Example 2) is commercially available from Clontech Labortories (Mountain View, CA); *E. coli* XL1-blue (Example 1) is commercially available from Stratagene Cloning Systems (La Jolla, California); and plasmid pUC18/p40 (Example 3) is commercially available from Cytokine bank (http://cytokine.chonbuk.ac.kr/main3.htm).

Accordingly, the specification enables one skilled in the art to make and use the claimed invention. It is respectfully requested that the rejection be withdrawn.

D. Rejection of Claims 1 and 4-9 under 35 U.S.C. 35 U.S.C. § 102

Claims 1 and 4-6 stand rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Jeong *et al.* (1999) or Martens *et al.*

Jeong *et al.* was relied on to disclose the expression of human leptin in *E. coli*, which endogenously contain a *cysK* gene. Martens *et al.* is replied upon to teach the expression of porcine IL-12 P40 subunit in *E. coli*, which endogenously contain a *cysK* gene.

Claims 7-9 stand rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Seibelt *et al.* (WO 03/006666) ("Seibelt"). Seibelt was relied upon to teach *E. coli* transformed with an *E. coli cysK* gene and a heterologous gene.

Claims 1, 7 and 9 have been amended to change the term "foreign protein" to "serine-rich protein." As such, the rejection under 35 U.S.C. § 102 becomes moot and it is respectfully requested that the rejection be withdrawn.

E. Rejection of Claims 1-9 and 11-14 under 35 U.S.C. 35 U.S.C. § 103

Claims 1-9 and 11-14 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Ramirez *et al.* and Lamouse-Smith *et al.* in view of Martens *et al.*, Swiss-Prot Accession No. P29460, Hatomoto *et al.* (JP 09/009982) and Koonin *et al.* Applicants respectfully traverse.

Remirez *et al.* was relied upon to teach that a recombinant protein production in *E. coli* can be improved by increasing the available levels of amino acids present in the recombinant protein in levels substantially above the levels of that amino acid in *E. coli.* Lamouse-Smith *et al.* also was relied upon to disclose that when an amino acid is present in a recombinant protein at levels significantly higher than present in host cellular protein, the amino acid becomes a limiting factor in expression level and that the metabolic burden imposed by amino acid composition can be alleviated by supplementing the cell with required precursors, leading to significant increase in recombinant protein expression. Lamouse-Smith allegedly teaches a negative correlation between expression level and cysteine content relative to the host.

Martens *et al.* was relied upon to teach the usefulness of IL-12 p40.

Swiss-Prot Accession No. P29460 was relied upon to teach the amino acid sequence of human IL-12 p40 showing that the protein has 3.3% cysteine residue, while Koonin *et al.* allegedly teaches that the average *E. coli* proteins have 1.1% cysteine residues. Hatomoto was relied upon to teach a method of increasing the amount of cysteine produced in *E. coli* using a plasmid encoding *cysE*, *cysK* and *pta* genes.

The Office Action asserted that it is known that IL-12 p40, which is a serine-rich protein, has a higher cystein residues than average amino acid composition of *E. coli* proteins and

Hatomoto teaches a method of producing increased cysteine, and, therefore, the one skilled in the art would be motivated to use Hatomoto's cells (having a plasmid comprising *cysK* gene) to produce increased amount of IL-12 p40, which is useful as an IL-20 antagonist for the treatment of septic shock.

First, Applicants would like to point out that the amount of cysteine in a host cell is not a main factor for increasing production of serine-rich protein. Rather, metabolic burden in synthetic pathway of serine family amino acids in the early stage of serine-rich protein production prevents the synthesis of serine-rich proteins. (*See* paragraphs [0030], [0031], [0037] in the present application).

cysK gene is associated with cysteine synthetic pathway. However, it cannot increase cysteine production. The main factor for increasing cysteine production is *cysE* gene and the amount of cysteine in cells is automatically regulated by feedback inhibition. If the amount of cysteine is excessive, it is degraded by degradation pathway.

Applicants found that amplification of *cysK* gene activated the synthetic pathway of whole serine family amino acids (glycine, serine, cysteine). Applicants also identified that the amplification of *cysE* gene in the cells by, for example, introducing *cysK* gene did not have an effect on the production of serine family rich proteins.

All of Jeong et al., Marten et al., Seibelt et al., and Ramirez et al. teach the increase of foreign protein productions by adding various amino acids to a culture solution. Since amino acids are expensive, these methods are not cost-effective. Furthermore, in the case of specific protein production, an excessive amount of amino acids inhibits the protein production.

Therefore, these methods are disadvantageous because an optimum concentration of a specific amino acid should be determined passively.

In particular, Hatomoto teaches the use of a recombinant vector containing *cysK* gene, *cysE* and *pta* instead of the addition of *acetylcoA* in the production of L-sulfur containing amino acid. Hatomoto does not use *cysK* alone. Hatomoto does not teach or suggest a production of serine-rich proteins using a *cysK* gene.

The *cysE* gene is known as a most important gene in cysteine production. However, the applicants verified that the amplification of *cysE* gene in a host cell does not have any effects on the serine-rich protein production.

As can be seen in Example 5 of the present application, hG-CSF has the amount of cysteine more than two times average *E. coli* proteins; however, production of serine-rich protein did not increase. Leptin has the same amount of cysteine as average *E.coli* protein; however, the production of serine-rich proteins increased largely. Therefore, it is considered that the increase in the amount of cysteine alone does not assure an increase in the amount of serine-rich proteins expressed.

In addition, human serine-rich protein is an important and useful protein involved in cellular signaling pathway, such as kinase, membrane protein. However, it is reported that serine-rich protein is difficult to be produced in *E. coli* (Cheryl Bula and Kent W. Wilcox, "Negative Effect of Sequential Codons on Expression of Foreign Genes in *Escherichia coli*", Protein Expression and Purification, 7:92-103 (1996)) ("Bula"). A copy is attached hereto as Exhibit A. Bula describes that serine-rich domains having long serine codons is poorly

expressed in *E. coli.*; however, the exact cause has not revealed yet. Bula showed that generally known transcription terminations, mRNA stability, proteolysis and codon usages are not a main cause of a difficult expression of serine-rich protein; and the production of serine-rich protein is reduced in proportion to the numbers of serine codons. Therefore, the difficulty of serine-rich protein productions in *E.coli* was well known at the time of the filing of the present application. Applicants could increase the production of serine-rich proteins by introducing *cysK* gene.

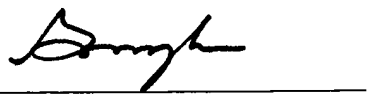
Applicants identified problems associated with a synthetic pathway of serine-rich amino acids using proteome research, and selected *cysK* gene among many candidates to perform recombinant gene manipulations, thereby accomplishing the present invention. Also, applicants identified that the amount of cysteine was not a main factor in a synthetic pathway of serine-rich proteins from the results obtained by amplifying *cysE* gene in host cells.

None of references cited by the Office Action teaches or provide suggestion to accomplish the presently claimed invention with a reasonable expectation of success. Therefore, it is respectfully requested that the rejection be withdrawn.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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Date: June 26, 2006

Attachment: Exhibit A.